Better together or single by choice? Challenges and opportunities in engineering microbial consortia for industrial biomanufacturing applications

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Better together or single by choice?

Challenges and opportunities in engineering microbial consortia for industrial biomanufacturing applications

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Microbial diversity

Known species

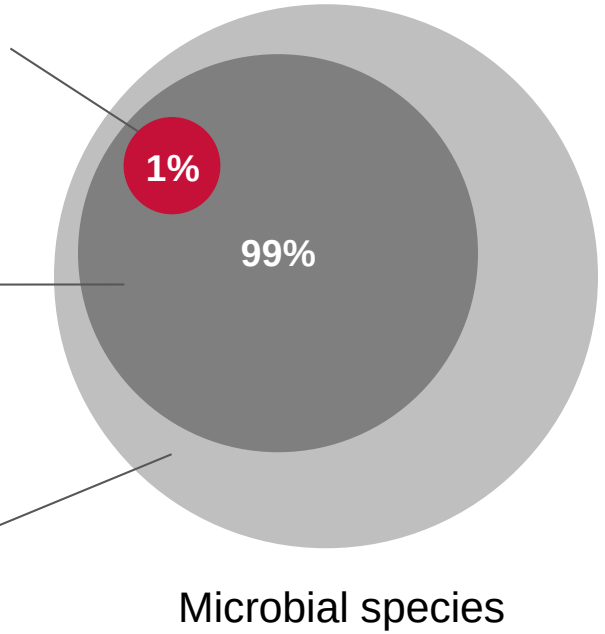
DSMZ, one of the largest bio-resource centers worldwide, contains some 10 000 bacterial species[1].

Unknown species

DNA sequencing of environmental samples reveals up to 100 000 bacterial species in a *single gram* of soil[2].

Unexplored

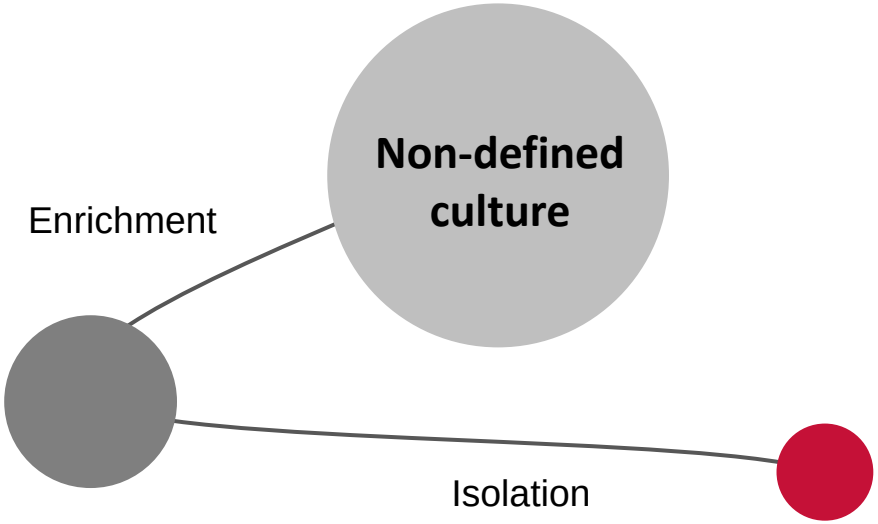
Non-quantifiable



How to tap nature's full potential?

[1] DSMZ self-description; https://www.dsmz.de/catalogues.html, accessed 22.10.2018
[2] Roesch, Luiz FW, et al. "Pyrosequencing enumerates and contrasts soil microbial diversity." The ISME journal 1.4 (2007): 283.

Application roadmap



Mixed culture

- No risk of contamination
- Non-sterile substrates
- Better together?

Pure culture

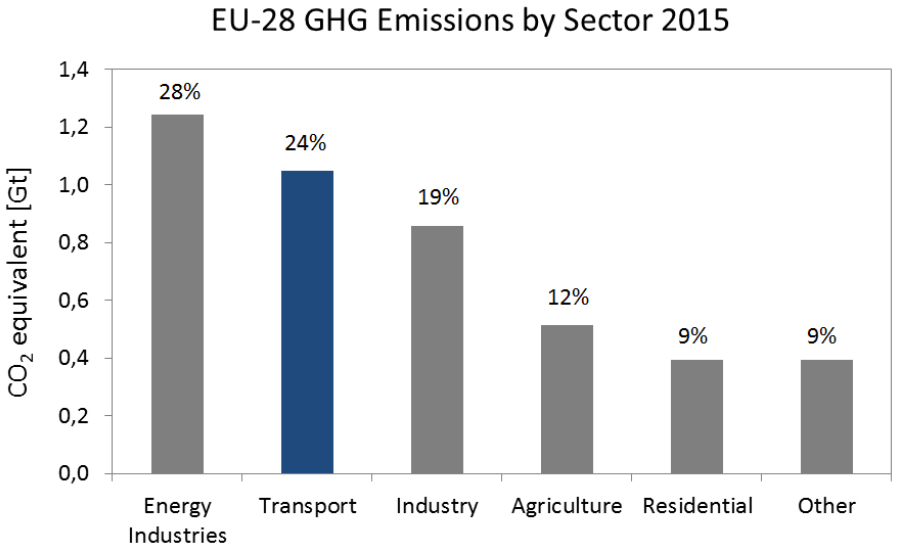
- New biochemical features
- New pathways
- Single by choice?

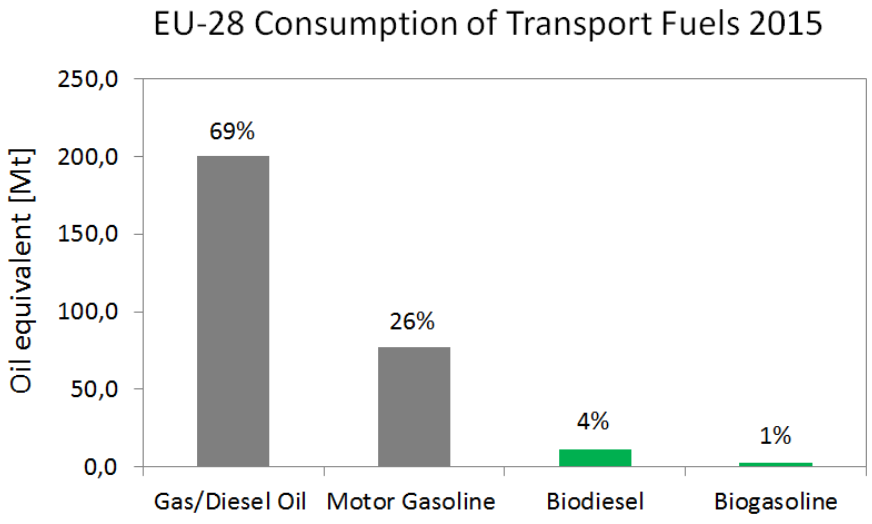
Two challenges

Butanol production

Keratin degradation

Need for sustainable biofuels





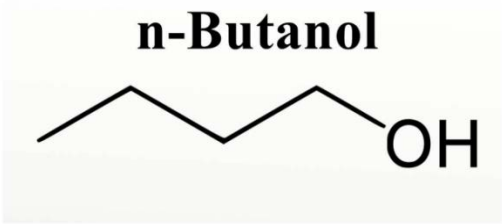
- In the EU, transport accounts for 24% of greenhouse gas (GHG) emissions[3]
- 95% of transport fuels are fossil-based[4]

[3] Eurostat [env_air_gge], EU-28 GHG emissions by source sector 2015, data source: European Environment Agency (EEA), 08-06-2017
[4] Statistical pocketbook 2017: EU transport in figures (PDF) p. 136, ISBN 978-92-79-62312-7, datasource: European Environment Agency (EEA), June 2017

The opportunity

Butanol

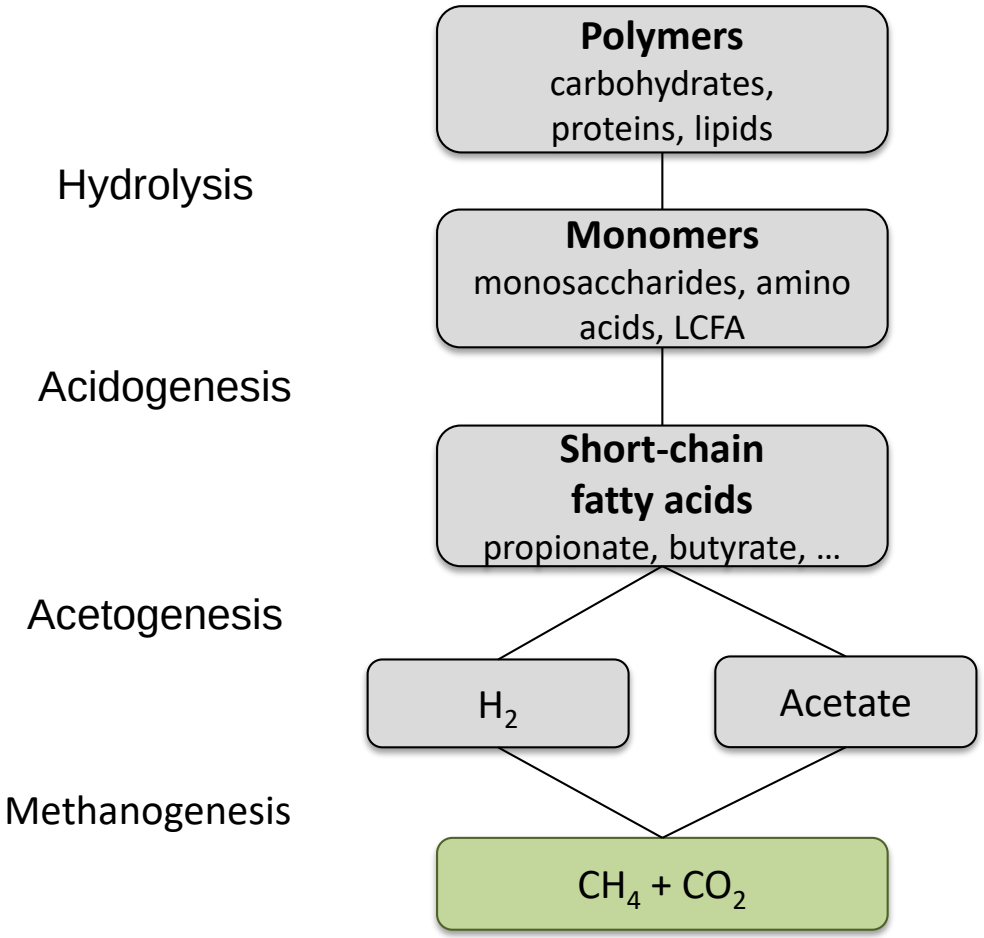
- Drop-in replacement for fossil transport fuels
- Properties similar to gasoline
- 50% higher energy density than ethanol



Mixed culture

- Use of non-sterile substrates
- Energy recovery from waste streams
- Closing the sustainability gap

Anaerobic digestion: Degradation view



Focus on biogas

Anaerobic digestion: Production view

Hydrolysis

Acidogenesis

Polymers
carbohydrates, proteins, lipids

Monomers
monosaccharides, amino acids, LCFA

Short-chain fatty acids
propionate, butyrate, …

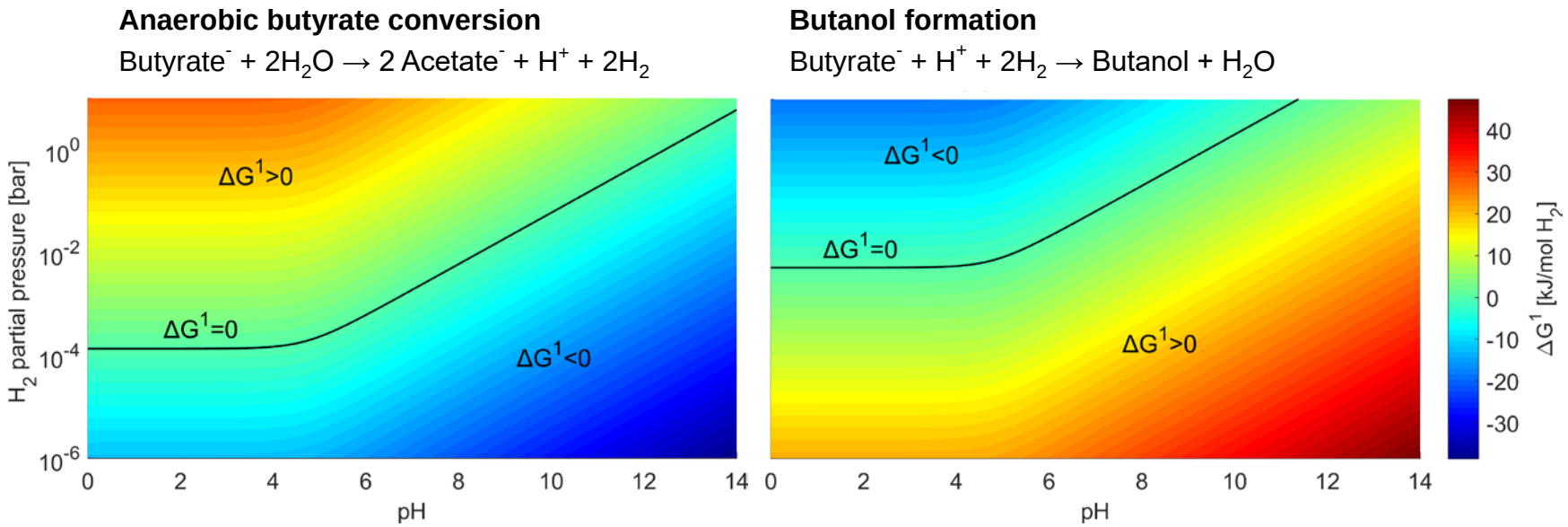
Extended range of green chemicals

- Butanol
- Bioplastics
- Hexanoate
- Heptanoate

✓ Non-sterile operating conditions
✓ Resource and energy recovery
✓ No competition with food production
✓ Low risk of functional loss

Enrichment strategy

- Thermodynamic engineering of anaerobic microbial consortia
- Operating under non-standard conditions (high H_2, low pH)
- Screening for butanol-forming species



Modelling results

Butanol Productivity

Energy recovery

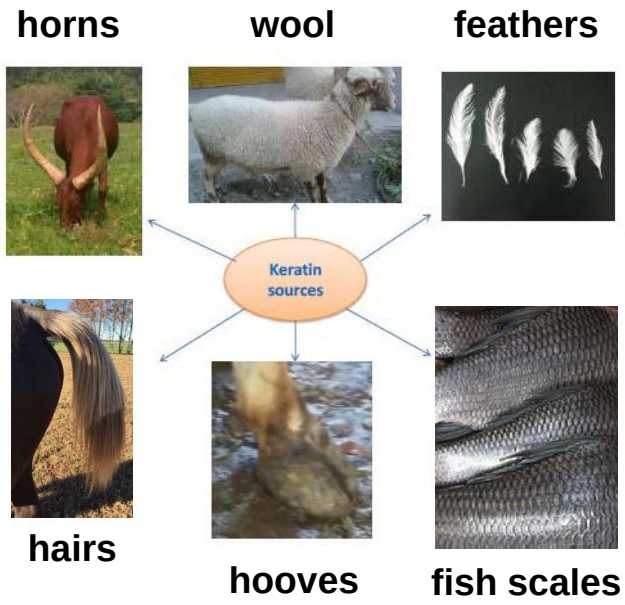
Confidential information on this slide has been removed.

Conclusions

- Thermodynamics as a powerful tool for microbial community engineering when reactions proceed close to thermodynamic equilibrium
- Modelling of an anaerobic full-scale, continuously stirred tank reactor showed that 10% of influent COD can be channeled towards butanol formation
- Results suggest a two-stage process for butanol/methane production

Keratinous Waste

Keratins are highly specialized fibrous structural proteins

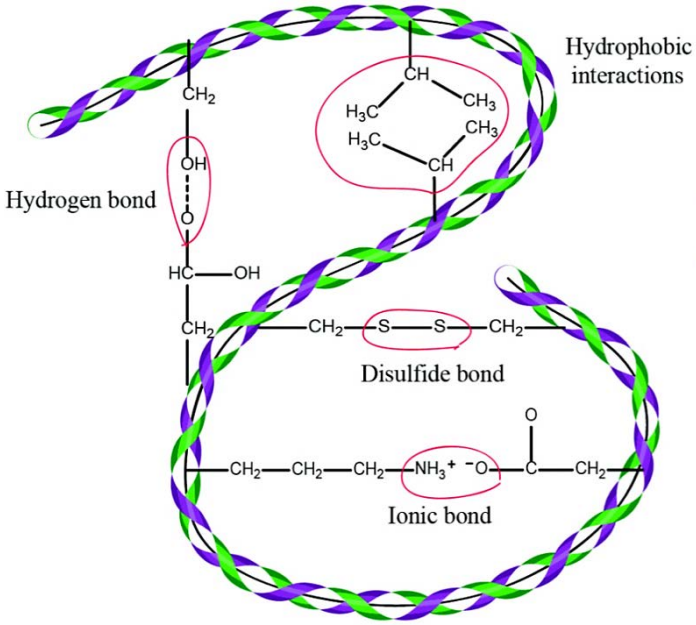


Adapted from:
Sharma, S., Gupta, A., 2016. Sustainable Management of Keratin Waste Biomass: Applications and Future Perspectives. Brazilian Arch. Biol. Technol. 59.

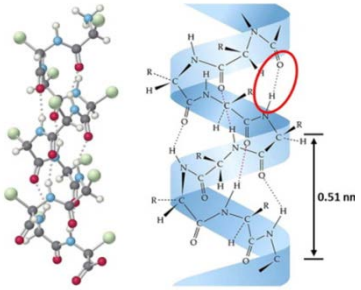
Keratin-rich animal by-products

originate from a wide range of wastes which are especially abundant in slaughterhouses and meat & poultry processing plants

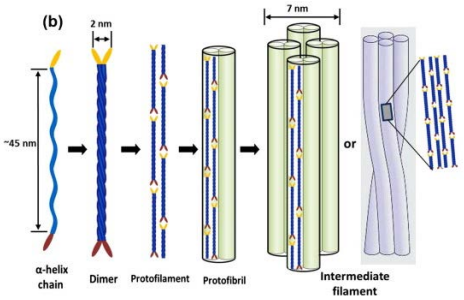
Keratinaceous Proteins



α-helix



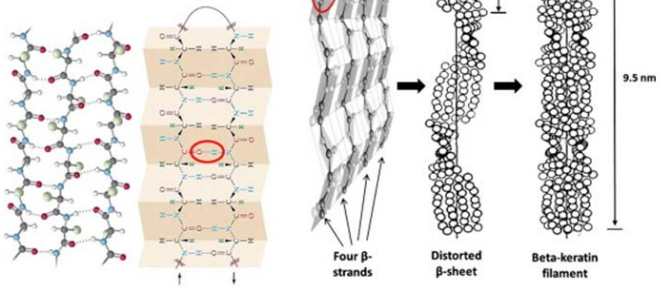
α-type



pig hairs

β-type

β-sheet



chicken feathers

Shavandi, A., Silva, T.H., Bekhit, A.A., Bekhit, A.E.D.A., 2017. Keratin: Dissolution, extraction and biomedical application. Biomater. Sci. 5, 1699–1735. https://doi.org/10.1039/c7bm00411g

Keratins are insoluble in water, organic solvents and weak acids and alkali and are resistant to common proteolytic enzymes such as papain, trypsin and pepsin

Porcine Bristles (pig hairs)

wet pig bristles (EU28 - 2017) ~ 225,000 tons

https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_animals#Livestock_population

Approx. 80% of this by-product consists of keratinic proteins

Approx. 60 k-tons of dry proteins

- ☹ **Logistical problem for disposal**
- ☹ **Significant protein chain loss**
- ☹ **Inefficient by-product use**

Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) https://eur-lex.europa.eu/eli/reg/2009/1069/oj

Category 1 High risk	Category 2 High risk	Category 3 Low risk
It includes carcasses/body parts of animals dead with transmissible spongiform encephalopathy (TSEs), contagious diseases, experimental procedures, illegal treatments etc. Includes also international catering wastes and died zoo/circus animals.	It includes carcasses/animals having infectious diseases, under authorized treatments and dead livestock. It also includes digestive tract materials of animals as well as died poultry in egg shell.	It includes animal skin, hooves, feathers, wool, horns, hairs etc with no signs of infectious agents. They also include eggs and related products as well as animal body parts at slaughterhouse meant for human consumption. This category of ABPs is usually reutilizable for various purposes of human economic interests.

*ABPs include animal carcasses, their parts or other materials derived from them which are unfit for human consumption but can be utilized for other purpose viz composting, bioenergy etc.

Verma, A., Singh, H., Anwar, S., Chattopadhyay, A., Tiwari, K.K., Kaur, S., Dhilon, G.S., 2017. Microbial keratinases: industrial enzymes with waste management potential. Crit. Rev. Biotechnol. 37, 476–491.

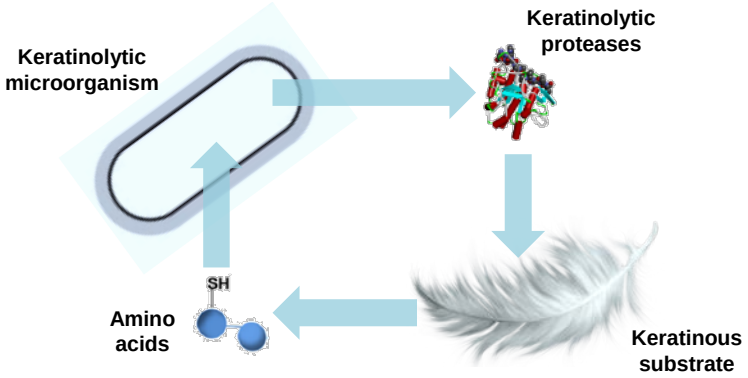
Microbial Degradation of Keratin

Bacteria, Actinomycetes and Fungi

Native keratin as only source of C, N, & Energy

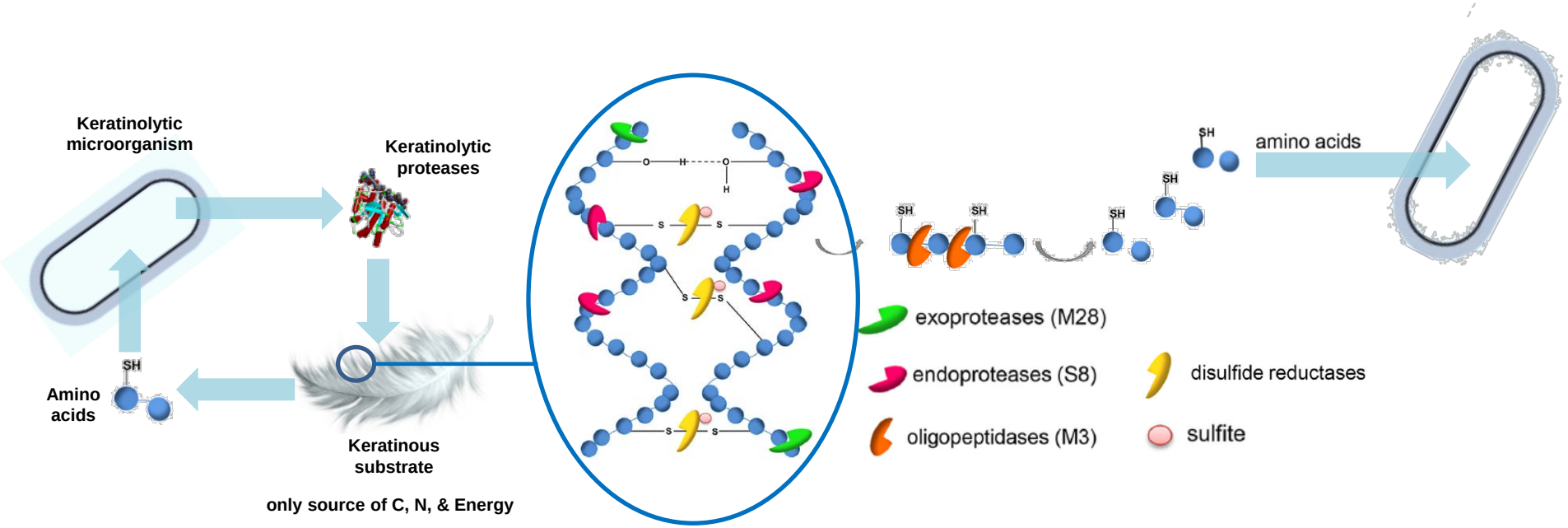
Keratinolytic microorganism → **Extracellular keratinases** → **Keratin waste decomposition**

Keratinases are enzymes belonging to the class of serine proteases or metalloproteases showing high levels of hydrolytic activity towards keratinous substrates



Keratin Degradation Mechanism

a cocktail of, at least, three microbial keratinases: an endoprotease (S8), an exoprotease (M28), and an oligopeptidase/metalloprotease (M3) may act synergistically to break down keratin



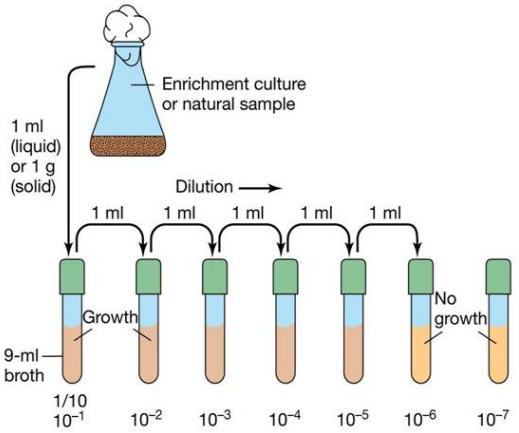
Lange, L., Huang, Y., Busk, P.K., 2016. Microbial decomposition of keratin in nature – a new hypothesis of industrial relevance. Appl. Microbial Biotechnol. 5, 2083-2096.

The Keratinolytic Strain

Int J Syst Evol Microbiol. 2003 May;53(Pt 3):871-4.

Amycolatopsis keratiniphila sp. nov., a novel keratinolytic soil actinomycete from Kuwait.

Al-Musallam AA[1], Al-Zarban SS, Fasasi YA, Kroppenstedt RM, Stackebrandt E.



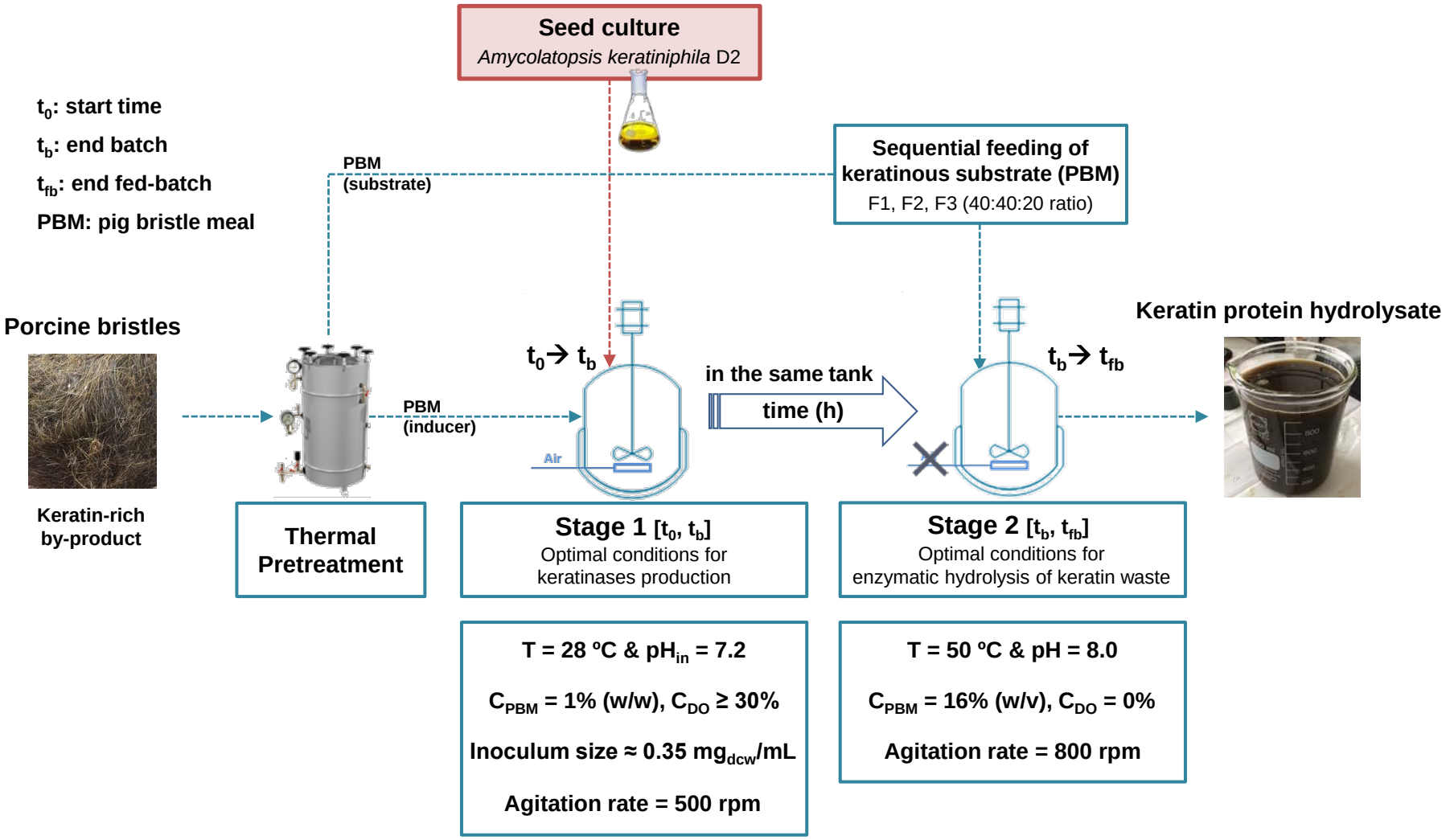
DSMZ

Amycolatopsis keratiniphila D2 (DSM 44409)

Gram+, aerobic obligated, mesophilic, filamentous bacterium

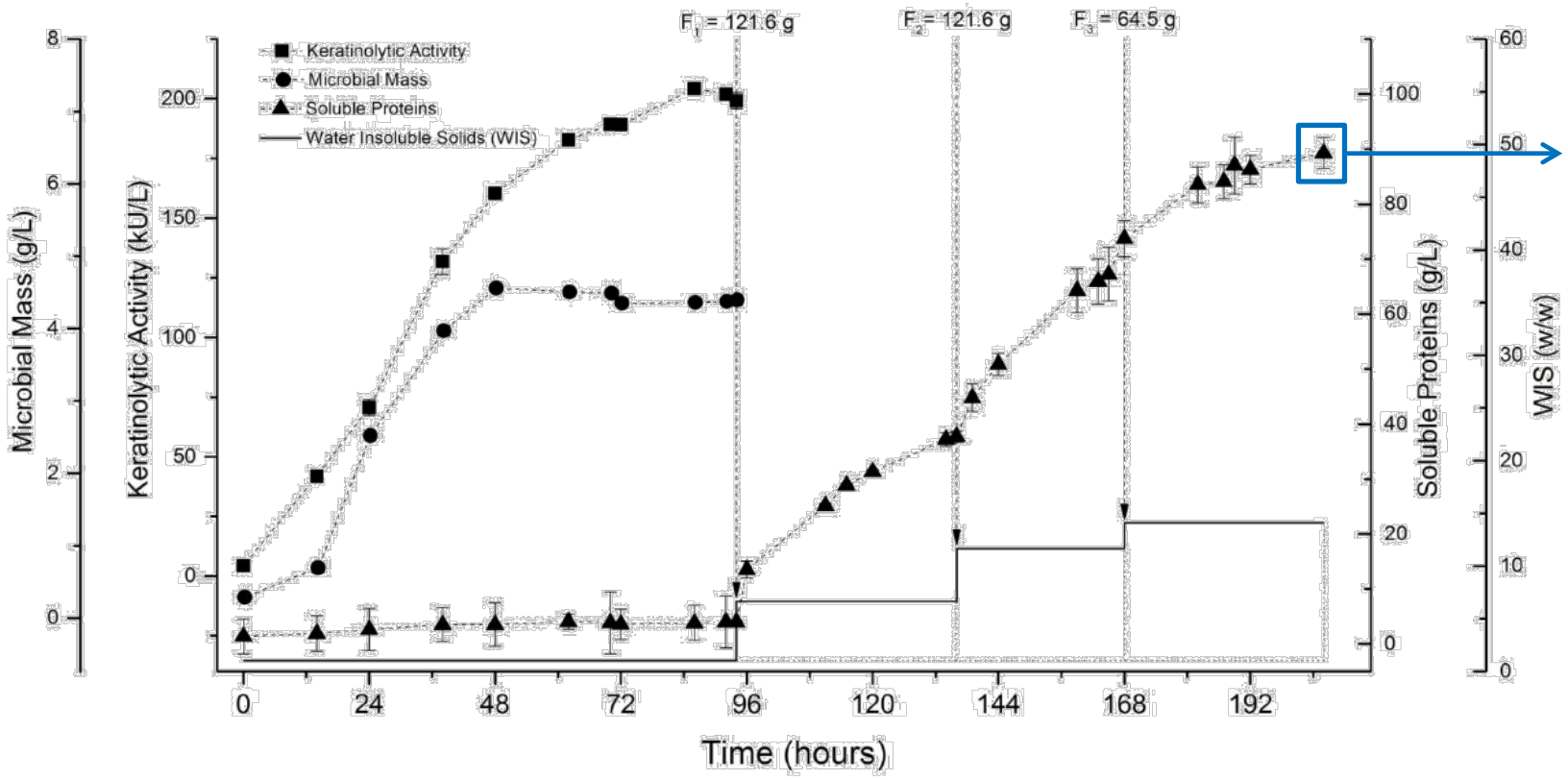
Two-Stage Cultivation Strategy

Amycolatopsis keratiniphila D2 (DSM 44409)



Two-Stage Cultivation Strategy

Amycolatopsis keratiniphila D2 (DSM 44409)



PBM_{LOAD}	164.3 $g \cdot L^{-1}$
$Y_{Extraction}$	72.5 %
STY	0.427 $g \cdot L^{-1} \cdot h^{-1}$

Conclusions

Amycolatopsis keratiniphila D2 was successfully employed for the **consolidate bioprocessing** of thermally pretreated pig bristles to protein-rich hydrolysates.

The **two-stage cultivation** approach constituted an effective way of obtaining high process performance matrices during hydrolysis of keratinic biowaste at high solids loading.

Thank you for your attention!

Project partners:

Keratin2Protein